Previously, using single-particle tracking at a temporal resolution of 0.025 ms, employing a 40-nmΦ colloidal gold probe, we have shown that virtually all of the lipid and protein molecules incorporated in the plasma membrane undergo hop diffusion. Based on this and many other observations, we proposed a model in which the entire plasma membrane is parcelled up into apposed domains due to the presence of the actin-based membrane skeleton (fence) and its associated transmembrane proteins (pickets), and membrane molecules undergo short-term confined diffusion within a domain (compartment), and long-term hop diffusion between the compartments. However, due to technological limitations, the observation of hop diffusion was only possible with a 40-nmΦ-colloidal gold probe, which might artifactually induce hop diffusion. To circumvent this problem, here, we developed a new high-speed, high-sensitivity CMOS camera system, which allowed us to track single fluorescently (0.5-nmΦ)-labeled molecules at a temporal resolution of 0.1 ms, the fastest single fluorescent-molecule imaging ever made. This camera system gave the position determination accuracy for single fluorescent molecules of ≈35 nm at a 0.1-ms time resolution. Virtually, all molecules of a phospholipid (DOPE) and a transmembrane protein, transferrin receptor, were found to undergo hop diffusion over the 110-nm compartments with median residency times of 9 ms and 33 ms, respectively, in the plasma membrane of a human epithelial T24 cell line. Meanwhile, in the actin-depleted, blebbed membrane, all of the DOPE and transferrin receptor molecules exhibited simple-Brownian diffusion. These results are in an excellent agreement with the previous high-speed gold-particle tracking data, and clearly indicate the necessity for the paradigm shift for the plasma membrane structure and dynamics, from the single continuous fluid model to the partitioned fluid model.

1594-Pos
Cluster Size Formation and its Effect on Protein Sorting in the Immunological Synapse
Niña C. Hartman[1], Jeffrey A. Nye[1], Cheng-Han Yu[1], Wan-Chen Lin[1], Jay T. Groves[1,2].
[1]UC-Berkeley, Berkeley, CA, USA, [2]Howard Hughes Medical Institute, Berkeley, CA, USA.
Micron-scale assemblies of molecules is thematic in biology, although oftentimes their exact function and mechanism of formation are unknown. A hallmark example is the immunological synapse (IS). T cell detection of pathogenic invasion on an antigen-presenting cell leads to the arrangement of receptor-ligand pairs into well-defined concentric zones. Specifically, T cell receptors (TCR) bound to peptide-presenting major histocompatibility complex (MHC), occupy the central zone surrounded by a ring of leukocyte function associated antigen-1 (LFA-1) bound to intercellular adhesion molecule-1 (ICAM-1). We postulate that the differences in cluster sizes between large TCR:pMHC micro-clusters and small LFA-1:ICAM-1 complexes prior to centripetal actin transport determine their differential sorting. To study this, we increase the LFA-1 cluster size two additional degrees beyond its native state by crosslinking LFA-1 or ICAM-1 on the supported membrane with a bivalent or tetravalent crosslinker. Progressively more central localization of LFA-1 proportional to the degree of crosslinking results until LFA-1 occupies the central zone with TCR. The different clustering states are identified using fluorescence correlation spectroscopy (FCS). Furthermore, the addition of a costimulatory interaction also increases radial transport. Thus, we demonstrate that the well-regulated event of clustering is a critical parameter in determining spatial patterning in the IS. We propose a sorting mechanism based on frictional protein coupling to actin, which is consistent with our observations and may be generalized to all membrane proteins in the IS.

1595-Pos
Flow Induced Protein Reorganization on Cell Surfaces
Eric Stellamanns[1], Sravanti Uppaluri[1], Niko Heddergott[2], Markus Engstler[2], Thomas Pfohl[3,1].
[1]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany, [2]Department of Zoology, University of Würzburg, Würzburg, Germany, [3]Department of Chemistry, University of Basel, Basel, Switzerland.
Living in an environment of continuous flow the unicellular human bloodstream parasite Trypanosoma brucei utilizes shear forces to remove hostile antibodies from its surface. African trypanosomes are spread via the bite of the Tsetse fly and cause the so called sleeping sickness in human and various diseases in animals as well. Their motility is driven by a single flagellum attached along the whole length of the cell and is essential for survival within the hostile immune system.
We emulate the bloodstream environment with PDMS microfluidic devices by means of flow velocity, shear gradients and confinement to examine the effect on trypanosome surface protein sorting and motility. Using highly fluorescent quantum dots (Qdots) to mimic antibodies and optical tweezers to control the cells, we are able to observe protein sorting in real time in order to quantify hydrodynamic drag driven protein reorganization in living cell membranes.

1596-Pos
Different Types of Lateral Diffusion Measurements Reveal that Unlike HA, Dc-SIGN is Immobilized in Microdomains
Michelle S. Itano[1], Aaron K. Neumann[1], Feng Zhang[2], Wolfgang J. Parak[2], Nancy L. Thompson[1], Ken Jacobson[1].
[1]University of North Carolina, Chapel Hill, NC, USA, [2]Philipps Universitat Marburg, Marburg, Germany.
Hemagglutinin (HA), from influenza virus, and DC-SIGN, a dendritic cell C-type lectin that binds many pathogens including viruses, bacteria, and fungi, form microdomains on the plasma membrane. We investigated the dynamics of these proteins using Scanning FCS (S-FCS), defined valency quantum dot-based Single Particle Tracking (SPT) and Fluorescence Recovery After Photobleaching (FRAP). Using FRAP we verified that HA has a large mobile fraction (~80%) that is characterized by a diffusion coefficient of ~0.09 um^2/sec and that it exchanges with the surround (Ellens et al. (1990) *Biochemistry,* **29**(41): 9697-9707). By contrast, neither DC-SIGN or Δ35-DC-SIGN, a DC-SIGN mutant missing the cytoplasmic tail, recovered after photobleaching, even after many minutes, indicating that these molecules do not exchange significantly with the surround. In order to determine the dynamics of molecules within the domains, we utilized a confocal line S-FCS method that permits the autocorrelation function between the same pixel in successive linescans (acquired in the form of a carpet or kymograph) to be calculated on the ms timescale. HA lateral motion within its microdomain is characterized by a diffusion coefficient of ~0.10 um^2/sec, similar to that measured outside the domain by FRAP. On the other hand, DC-SIGN and Δ35-DC-SIGN do not diffuse within the domain. We are currently using quantum dots that have been conjugated to a *single* streptavidin in order to examine lateral dynamics within these domains. Such nanoparticles will obviate a consistent interpretative limitation for gold and quantum dot SPT; namely, that particle valency cannot be specified with absolute certainty for the particle whose motion is tracked. Further characterization of these surprisingly stable DC-SIGN domains that are important for pathogen entry into dendritic cells is in progress.
Supported by NIH GM 41402 (KJ &NLT)

1597-Pos
Psychostimulants Affect Dopamine Transporter Lateral Mobility and Membrane Microdomain Distribution
Jeffrey S. Goodwin[1], Tina Patel[1], Anne K. Kenworthy[2], Habibeh Khoshbouei[1].
[1]Meharry Medical College, Nashville, TN, USA, [2]Vanderbilt University, Nashville, TN, USA.
Neurotransmitter reuptake by transporters is a major mechanism for terminating synaptic transmission. The human dopamine transporter (hDAT) is one of the main targets for psychostimulants, and is critical to DA homeostasis. Lipid rafts are specialized membrane microdomains that serve as organizing centers to regulate different cellular processes such as neurotransmission and trafficking. To begin to understand how psychostimulants, methamphetamine (METH) and amphetamine (AMPH), affect hDAT microdomain association, we utilized fluorescence recovery after photobleaching (FRAP) and density-gradient centrifugation. Our FRAP studies revealed significant changes in the rate (D) and extent (Mf) of the fluorescence recovery into the bleached region of the plasma membrane in cells expressing YFP-hDAT in the presence of METH but not AMPH. Substitution of five N-terminal Ser with Ala, (cannot be phosphorylated) or Asp (pseudo-phosphorylated), and removal of the 22 N-terminal amino acids restored the diffusion rate of the transporter to control levels. Using density-gradient centrifugation, we found that YFP-hDAT is distributed into both, classically defined, membrane raft and non-raft fractions. Incubating with METH and AMPH shifted YFP-hDAT from non-raft to raft fractions. We have previously shown that METH uniquely modulates the biophysical properties of DAT. Our present findings suggest that METH and AMPH cause hDAT to partition into lipid raft membrane microdomains, and the decrease in the hDAT diffusion rate evoked by METH- vs. AMPH-occupied DAT could suggest that the N-terminal domain of the transporter is associated with a distinct group of proteins when exposed to these psychostimulants, and may thus describe an underlying mechanism behind the addictive biological differences between these psychostimulants.